DIVERSITY, PREVALENCE AND HOST SPECIFICITY OF PARASITES IN FRESHWATER FISH POPULATION IN SELECTED RESERVOIRS OF PERAK RIVER, PERAK

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by

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TABLE OF CONTENTS

LIST OF TABLES

Page

LIST OF FIGURES

LIST OFABBREVIATIONS

Kn	Relative Condition factor
cm	centimetre
mg/L	milligram/litre
km	kilometre
μL	microliter
^{o}C	Degree Celsius
%	Percentage
sp.	Species
mm	millimetre
μm	micrometre
bp	base pair
g	gram
TL	Total length
r	Pearson correlation coefficient

LIST OF APPENDICES

KEPELBAGAIAN, PREVALENS DAN KEKHUSUSAN PERUMAH PARASIT DALAM POPULASI IKAN AIR TAWAR DI KAWASAN EMPANGAN TERPILIH DI SUNGAI PERAK, PERAK

ABSTRAK

Hasil kajian yang terdahulu dan terkini telah menunjukkan bahawa terdapat banyak penyakit patogen yang disebabkan oleh parasit dalam ikan tetapi banyak jenis parasit ikan yang masih belum dikenal pasti. Tiada maklumat mengenai prevalens fauna parasit protozoa dan myxozoa ikan di sepanjang Sungai Perak. Kaedah pengecaman berdasarkan molekul adalah kaedah yang tepat bagi mengenalpasti jenis parasit tiada jujukan DNA parasit ikan yang pernah dijalankan dari semua empangan di sepanjang Sungai Perak. Kajian ini telah mengenal pasti parasit ikan dari April 2017 hingga Mac 2019 di empangan Temengor, Bersia, Chenderoh kecuali empangan Kenering, Perak. Morfologi dan pengenalpastian melalui kaedah molekul buat pertama kali bagi beberapa monogenean terpilih telah dikaji. Interaksi perumah-parasit kemudiannya digambarkan oleh graf bipartite, diikuti dengan analisis kesan parasit ikan terhadap faktor keadaan relatif (Kn), dan hubungan parasit dengan suhu air, pH, dan oksigen terlarut (DO) takungan. Sejumlah 276 ekor ikan disampel, didapati 161 (58%) ekor ikan mengandungi parasit. Tujuh belas daripada 25 spesies ikan mengandungi parasit. Tiga puluh dua spesies parasit ikan telah dikenalpasti dalam kajian ini yang merangkumi dua spesies protozoa, dua spesies myxozoa, dua belas spesies monogenea, tiga spesies digenea, satu spesies sestod, empat spesies nematod, tiga spesies akantosefala dan lima spesies krustasea. *Balantidium* sp., *Henneguya* sp. dan *Myxobulus* sp. adalah spesies parasit baru yang direkodkan di dalam empangan. Parasit dominan yang terdapat di dalam ikan adalah monogena dengan *Paradiplozoon barbi* (17.39%) merekodkan kadar prevalens tertinggi. Kajian ini telah mengenalpasti

Dactylogyrus lampam dan *P. barbi* dari takungan dengan menggunakan kaedah morfologi dan molekul. *Dactylogyrus lampam* dan *P. barbi*. Jujukan DNA monogenea rDNA 18S *D. lampam* dan ITS-2 region *P. barbi* telah dikenalpasti buat pertama kalinya. Jujukan DNA baru telah dilaporkan dalam pangkalan data GenBank dengan nombor akses MN617155 dan MN688771. Rangkaian interaksi perumah-parasit menunjukkan *P. barbi,* parasit umum yang terdapat pada ikan siprinid mencatatkan prevalens tertinggi manakala *Barbonymus schwanenfeldii* mencatatkan interaksi tertinggi dengan parasit. Kesan parasit ikan terhadap faktor keadaan relatif (Kn) ikan bergantung kepada kevirulenan parasit dan ketersediaan makanan. Nilai pH, DO dan suhu yang direkodkan dalam kajian ini adalah sesuai untuk kelangsungan hidup ikan. Korelasi parameter fizikal dengan parasit ikan menunjukkan bahawa hanya sista protozoa dan *Acanthocephalus* sp. menunjukkan korelasi positif yang signifikan dengan DO. Hanya beberapa spesies parasit ikan sahaja yang menunjukkan hubungan dengan pH, DO, dan suhu manakala spesies yang lain tidak. Majoriti parasit mempunyai korelasi yang lemah dengan parameter fizikal air kerana pengukuran kualiti air empangan yang tidak teratur semasa kajian dilaksanakan. Kesimpulannya, kajian ini mendedahkan adanya tiga spesies parasit yang baru dilaporkan di empangan. Parasit dominan yang diasingkan dari ikan adalah monogena. Urutan DNA beberapa parasit ikan diketahui untuk pertama kalinya. Pengaruh parasit terhadap kesihatan ikan ditentukan oleh kevirulenan parasit dan ketersediaan makanan kepada hos ikan. Sebilangan parasit ikan menunjukkan korelasi positif yang signifikan dengan nilai DO empangan di sepanjang Sungai Perak.

DIVERSITY, PRAVALENCE AND HOST SPECIFICITY OF PARASITES IN FRESHWATER FISH POPULATION IN SELECTED RESERVOIRS OF PERAK RIVER, PERAK

ABSTRACT

Past and present studies have shown that there are numerous pathogenic diseases caused by parasites in fishes but many fish parasites remain to be identified. There was no information on the prevalence of protozoa and myxozoa parasitic fauna of the fishes in the reservoirs along the Perak River**.** Molecular identification provides an accurate method for the identification of parasites. The DNA sequence of fish parasites in reservoirs along the Perak River has not been studied before. This research has identified the fish parasites from April 2017 until March 2019 in Temengor, Bersia, Chenderoh reservoirs except for Kenering reservoir, Perak. Morphological and first molecular identification of some selected monogeneans were studied. The host-parasites interactions were then visualized by a bipartite graph, followed up by the analysis of the effects of fish parasites on the relative condition factor (Kn), and the relationship of parasites with the water temperature, pH, and dissolved oxygen (DO) of the reservoirs. A total of 276 individuals fishes were sampled from the reservoirs with 161 (58%) fishes individuals were presence with parasites. Seventeen fish species were presence with parasites out of the 25 species. Thirty-two species of fish parasites were identified in this study which include two species of protozoans, two species of myxozoa, 12 species of monogeneans, three species of digeneans, one species of cestode, four species of nematodes, three species of acanthocephalans and five species of crustaceans. *Bolantidium* sp., *Henneguya* sp**.** and *Myxobulus* sp. were new recorded parasites species in the reservoirs. Monogeans were the dominant parasites found in the fishes with *Paradiplozoon barbi* (17.39%) recorded the highest prevalence rate.

This study has identified *Dactylogyrus lampam* and *P. barbi* in the reservoirs using morphological and molecular methods. The 18S rDNA of *D. lampam* and ITS-2 region of *P. barbi* DNA sequences of these monogeneans are known for the first time. The new DNA sequences were deposited in the GenBank database with accession numbers MN617155 and MN688771, respectively. The host-parasites interaction network showed *P. barbi*, a generalist parasite on cyprinids recorded the highest prevalence and *Barbonymus schwanenfeldii* recorded the highest interactions with parasites. The effects of fish parasites on the relative condition factor (Kn) of fish depend on parasites virulence and food availability. The value of pH, DO and temperature recorded in this study is suitable for fish survival. The correlation of physical parameters with fish parasites showed that only protozoan cyst and *Acanthocephalus* sp. showed a significant positive correlation with DO. Some fish parasites showed a relationship with pH, DO, and temperature, while others do not. Majorities of the parasites weakly correlated with physical water parameters due to the irregular measurement of water quality of the reservoirs during the study. In conclusion, this study revealed the presence of three newly reported parasites species in the reservoirs. The dominant parasites isolated from the fishes were monogeneans. The DNA sequences of some fish parasites were known for the first time. The influence of parasites on the fish health are determined by the parasite's virulence and food availability to the fish host. Some fish parasites showed a significant positive correlation with DO values of the reservoirs along Perak River.

CHAPTER ONE

INTRODUCTION

1.1 General introduction

In many parts of the world, fish is one of the sources of providing less expensive animal protein and also income for fishermen (Abdel-Gaber *et al*., 2015). Wild fish are the main source of fish production. The depletion of feral fish is a result of an increase in demand due to the rise in the human population, overfishing, habitat degradation, pollution, and parasitic diseases (Quiazon, 2015).

Freshwater fishes in the reservoirs can be exposed to stressful factors that predispose fish to diseases. Diseases of fish are also caused by environmental stress and the interactions between pathogenic organisms and fish hosts (Vasemägi *et al.*, 2017). The age of a reservoir is a vital factor influencing the burden of freshwater fish parasites, which is the time for fish parasites and fish hosts to colonize the reservoirs when created (Song & Proctor, 2020). Fishes in the reservoirs are subjected to many parasitic diseases due to poor quality of water and large-scale mortality is seen in the fish population due to parasitic diseases (Overstreet & Hawkins, 2017).

Despite advances in technological know-how to burst wild fish catch and fish farming production, yet a parasitic disease is a threat to food safety. Parasitic diseases are affecting wild fish populations by reducing the reproduction rate, slow growth, and surviving rates (Quiazon, 2015).

Apart from the pathogenic effect of fish parasites to wild and farmed fish, some fish parasites can cause health problems in humans, like herring worm diseases which are parasitic diseases caused by inactive stages of the third-stage larvae nematodes, liver flukes infection cause by metacercariae of trematodes and fish tapeworm infection caused by plerocercoid of tapeworms, which can be found in the fish flesh. These parasites are transmitted to humans after eaten poorly cooked or raw fish (WHO, 2015; Scholz & Kuchta, 2016**)**. Fish flesh eaten raw like sushi is recommended to be store at a temperature of -20 ºC for 7 days to kill any parasites or cooked fish at temperature 74 ºC for 2 minutes to kill any parasites (Robertson *et al.*, 2014).

Some parasites, bacteria, and viruses are pathogenic organisms that freshwater fishes are vulnerable to, these pathogens cause many disorders and diseases in freshwater fishes. Fish parasites have various means to infect the fish host which include depriving the fish host of enough food or vital substances like vitamins, localizing and outnumbering their population in vital organs of the fish host which leads to diseases (Iwanowicz, 2011).

Feral fish in the natural water bodies are usually parasitized by fish parasites which normally exist in equilibrium with the freshwater fish host, parasites may become a threat to the fish populations in the wild under an abnormal condition such as poor water quality (Goater *et al.*, 2014). An outbreak of diseases easily happened when fishes are confined under an overcrowded environment like in cultured fish farms, parasites and other pathogenic organisms are threats to farm fish rear on large scale **(**Assefa & Abunna, 2018). In fish culture and aquaculture operations, fish parasites are known to be a serious problem, thereby leading to the increase in the study of parasites that causes diseases in the fish parasitology group (Zajac *et al*., 2021).

Farm and wild fishes are parasitized by many organisms ranging from protozoans to myxozoans, monogeneans, digeneans, cestodes, nematodes, acanthocephalans, and crustaceans (Bahri & Marques, 2014; Goater *et al.*, 2014). Fish parasites derive their nutrients, enzymes, and oxygen from their host for their physiological requirements (Shea-Donohue *et al*., 2017). Parasitic infestations in culture fish lead to growth reduction, an increment of cost for treatment, and mortality (Roberts, 2012). Fish also serve as an intermediate host for parasitic larval forms that cause disease in vertebrates. Some parasites are host-specific, while others are generalist, the pathogenicity of parasitic diseases increases with intensity and prevalence (Roberts, 2012).

1.2 Problem statements

The number of parasites that are present in freshwater fishes are numerous, many more fish parasites remain to be identified and described (Roberts, 2012). There was no report of protozoa and myxozoa prevalence rate in the Perak River. The DNA sequence of monogeneans and other freshwater fish parasites in the reservoirs along the Perak River has not been studied. The number of monogeneans species identified is 10-20 % and many more are yet to be identified (Poulin, 2011). The use of morphometrics of the sclerotized copulatory organ, anchor, and clamp in identifying monogenean is problematic due to their variability in their size. The processing of histological tissues of this organism can deform the specimen orientation on a microscope slide. This may conceal the copulatory or anchor and the resolution of the compound microscope may be exceeded by the size of this organ as it reaches its limit. Some studies have been undertaken for several monogeneans species in the Perak River (Bu & Leong, 1997; 1999; Lim *et al.*, 2016). The DNA sequence of monogeneans and other freshwater fish parasites in the reservoirs along Perak River have not been studied before and identifying these parasites by genomic may prove a better way for the accurate identification of these numerous monogeneans. In this research, molecular and morphological methods were used to identify some monogeneans parasites in Temengor, Bersia, and Chenderoh reservoirs of Perak River.

Freshwater fish parasite prevalence is influenced by biotic factors and abiotic factors (Wali *et al*., 2016). The most important abiotic factors are temperature, dissolved oxygen and pH (Saha *et al.*, 2013). The length, weight, age, sex, host-parasites interactions and relative condition factor (Kn) of the fish are biotic factors that influence parasites' prevalence and distribution in the host **(**Vasconcelos & Tavares-Dias, 2016). There were no documented reports relating to fish-host parasite interactions, the influence of relative condition factor (Kn), and abiotic factors in freshwater fish parasitism in Temengor, Bersia and Chenderoh reservoirs. This research also aimed at investigating the influence of physical parameters, host-parasites interactions, and relative condition (Kn) factors on fish parasites in the reservoirs.

1.3 Justification of the study

Habitat degradation, overfishing and parasitic infection causes decrease in wild fish population and financial losses in culture fish. Fundamental biological variables like fish parasite composition baseline data, fish hosts-parasites interactions and environmental variables on fish parasites are needed in establishing a monitoring and management system of these freshwater fishes to mitigate the possible adverse effect of these parasitic diseases. Many parasitic organisms are yet to be identified that causes diseases in feral fishes. In comparison to the number of local and international journals on this matter, there is no information about protozoa and myxozoa parasitic fauna of the fishes in River Perak reservoirs.

The motivation of this current research was initiated due to the lack of published accounts of species of protozoa and myxozoa infection rates. Furthermore, there exist interaction between parasites, fish, and water quality in any aquatic ecosystem, with such interaction proving to be complex even within the ecosystem with few species. As a result, the possible influence on fish host-parasite interactions and environmental variables on fish parasite prevalence in River Perak reservoirs shall be studied.

1.4 Objectives of the study

The objectives of this research are as follows**:**

1. To identify the parasites from the freshwater fishes in Temengor, Bersia and Chenderoh reservoirs in Perak River and to study some selected species of monogeneans by morphological and molecular approaches.

2. To elucidate the fish host-parasite interactions using a bipartite network in Temengor, Bersia and Chenderoh reservoirs.

3. To determine and compare the effects of fish parasites and distribution with respect to relative condition factor (Kn) of the fish in Temengor, Bersia and Chenderoh reservoirs.

4. To investigate the relationship between physico-chemical parameters and parasitic infestation on freshwater fishes in Temengor, Bersia and Chenderoh reservoirs.

CHAPTER TWO

LITERATURE REVIEW

2.1 Perak River

Perak River is in Perak State, Malaysia, with a total length of 427 km approximately, making it the second-longest river in the Peninsular Malaysia (Hashim *et al.*, 2012). The river water source is Perak-Kelantan-Thailand mountainous border. Its water catchment areas include the Belum-Temengor Forest Reserve (Salam *et al*., 2019). The river flow from a watershed north to south along the state borders of Perak with Thailand, the state of Kelantan, and Kedah. The river is situated at a lowland with primary and secondary forest types of river systems. The depth of the river is about 0.9 m to 2.4 m. It is 100 m at the widest point and has a temperature range of 29.4 - 32.3 ^{o}C . The major towns along the river are Kuala Kangsar and Grik (Salam *et al*., 2019). Temengor reservoir is located on the upper most of the river follows by Bersia reservoir; located at about 20km downstream of Temengor reservoir. Next, Kenering reservoir; located at about 45 km downstream to Bersia reservoir. Chenderoh reservoir is the last reservoir (Salam *et al*., 2019).

2.2 Reservoirs along Perak River

Dams were constructed on Perak River which resulted in the creations of Chenderoh Reservoir in 1926, Temengor Reservoir in 1977, Bersia Reservoir in 1983 and Kenering Reservoir in 1983 respectively (Figure 2.1), for hydroelectric power generation, flood control, irrigation and domestic uses (Salam *et al*., 2019). The reservoirs of the Perak River provide the local communities with cheap animal protein and income to the fish farmers in the reservoirs (Abdel-Gaber *et al.,* 2015). A brief description of the reservoirs along Perak River are summarized in Table

2.2.1 Temengor Reservoir

Temengor Reservoir was created on Perak River which covered an area surface of 152 km^2 making it the second-largest reservoir after Kenyir Reservoir in Malaysia, it is located to the northeast of Gerik in Perak State. It is built on the upper Perak River immediately downstream of its confluence with the tributary Temengor River (Mohd & Pauzi, 2012).

The height of the Temengor dam is about 127 m which is a rockfill dam with a central impervious core creates a reservoir 80 km long with a catchments area of 340 km^2 . The dam impounds 6,050,000,000 m^3 of water at normal full supply level, of which 1,270,000,000 m^3 is utilized as live storage. The crest level of the dam provides sufficient freeboard for an additional 850,000,000 m^3 of flood storage capacity, which together with a free overflow chute spillway, will regulate flow past the dam during the flood period (Ezuria, 2002; Salam *et al.*, 2019).

2.2.2 Bersia Reservoir

Bersia Reservoir is another reservoir found on Perak River, it is about 20 km from Temengor Reservoir and 16 km from Gerik Town by road with an area surface of 5.7 km^2. The reservoir dam is a concrete gravity dam with a height of 33 m. The reservoir play a vital role in regulating water flow within the Perak River (Hashim *et al.*, 2012).

Bersia Reservoir have a catchments area of 3,560 km^2. The reservoir storage capacity is approximately 70, 000, 000 m^3 of water at normal full supply of which 58, 000, 000 m^3 of water is utilized as active storage. (Ezuria, 2002; Salam *et al*., 2019).

2.2.3 Kenering Reservoir

The Kenering Reservoir is located some 72 km downstream from Temengor Reservoir, about 45 km by road south of Gerik town. It is in a mountainous region about 40 km from Bersia Reservoir. The reservoir is made of a concrete gravity dam in the middle and a rock-filled dam on both sides. The reservoir is approximately 40 km^2 of surface area. The reservoir storage capacity is approximately 352, 000, 000 m^3 with a mean depth of 15 m (Hashim *et al*., 2012).

2.2.4 Chenderoh Reservoir

Chenderoh Reservoir is the oldest reservoir first created in Perak River. The reservoir is located 34 km north from Kualar Kangsar and 52 km toward Kenering Hydroelectric Power Station. Chenderoh Reservoir has a dendritic shape because of damming Perak River. The dam is about 390 m in length and 20 m in height respectively. The reservoir storage capacity is approximately 95, 000, 000 m^3 with a surface area of 21 km^2 and mean depth of 9 m (Hashim *et al*., 2012).

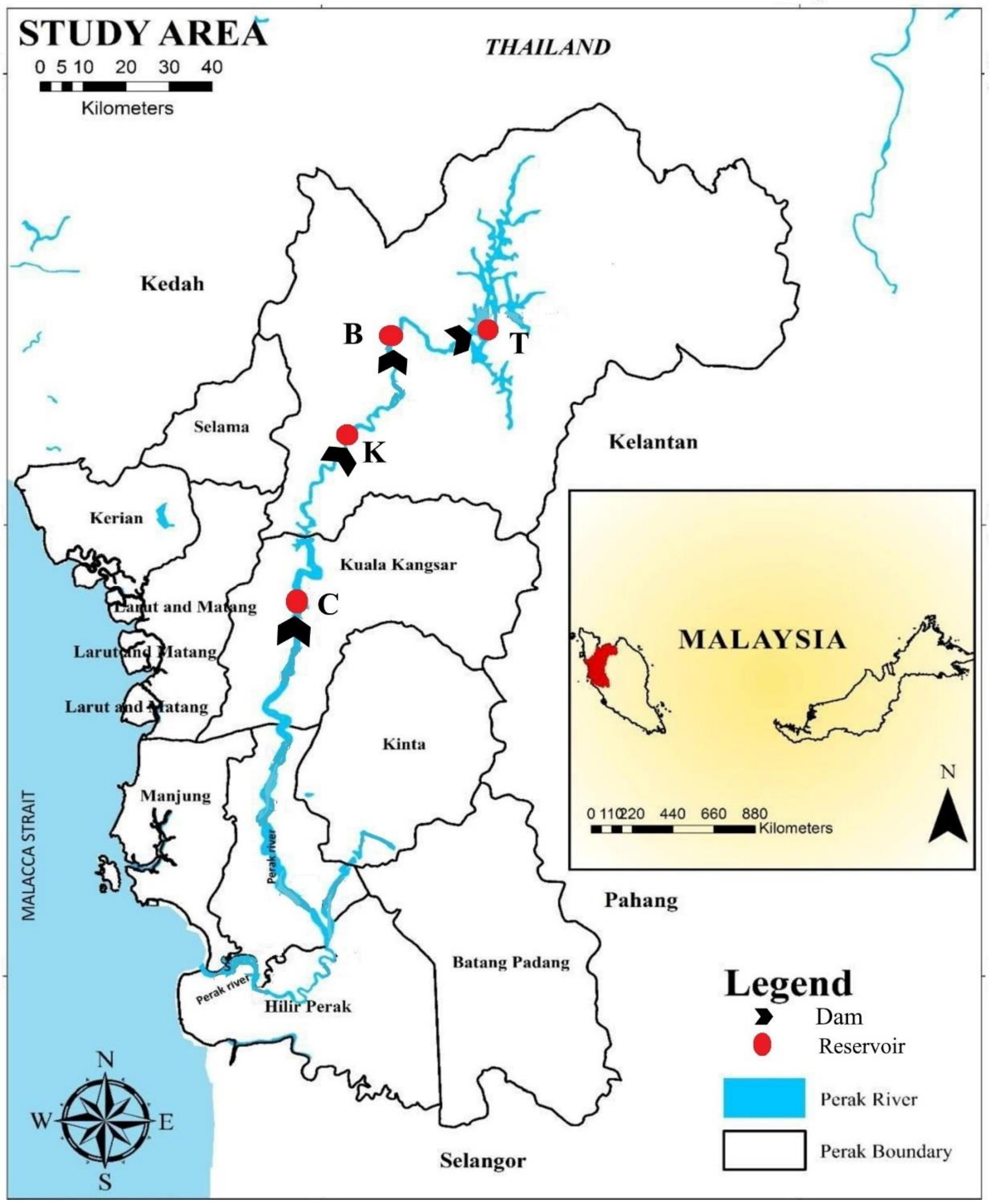

Figure 2.1 Map showing the location of Temengor (**T**), Bersia (**B**), Kenering (K), Chenderoh (**C**) Dams and Reservoirs along the Perak River. Adapted from Salam *et al*., (2019).

2.3 Freshwater fish commonly found in the reservoirs of Perak River

The global estimate of freshwater fish species is about 14,740 (Carrete & Wiene, 2012) which are found in reservoirs, rivers, streams, lakes, and ponds that occupy only 1% of the earth's crust. While marine fish comprises about 15,150 fish species that live in salt-water that covers 70% of the earth (Carrete & Wiene, 2012).

Malaysia has about 622 species of freshwater fish (Froese & Pauly, 2019). Hashim *et al.*, (2012) reported 107 fish species in reservoirs along the Perak River, which are dominated by Cyprinidae followed by Bagridae and Ariidae. *Hemibagrus nemurus, Barbonymus gonionotus, Osteochilus vittatus* and *Notopterus notopterus* are the species of fishes that are commonly found in the reservoirs. These fish species are highly adapted to changes in the physico-chemical parameters of the reservoirs. Table 2.1 shows the fish species commonly found in the reservoirs of Perak River (Hashim *et al.*, 2012; Zakaria-Ismail *et al.*, 2019).

Table 2.1 Fish species commonly found in the reservoirs along Perak River (Hashim *et al.,* 2012; Zakaria-Ismail *et al.,* 2019)

Fish species	Local name	Common name
Cyprinidae		
Osteochilus vittatus (Valenciennes,1842)	Rong Jawa	bonylip bard
Cyclocheilichthys apogon (Valenciennes,1842)	temperas Indonesia	beardless barb
Labiobarbus leptocheilus (Valenciennes, 1842)	Kawan Jakata	signal barb
Osteochilus melanopleura (Bleeker, 1852)	Rong Ralembang	kelabau carp
Puntioplites bulu (Bleeker, 1851)	tengalan banjarmasin	carp
Mystacoleucus obtusirostris (Valenciennes 1842)	sia Jawa	Blunt-snout barb
Hampala macrolepidota (Kuhl &Van Hasselt 1823)	sebarau Jawa	hampala barb
Barbodes binotatus (Valenciennes 1842)	tebal sisik Jawa	spotted bard
Cyclocheilichthys armatus (Valenciennes 1842)	temperas Jawa	beardless bard
Labiobarbus festivus (Heckel, 1843)	Kawan Kalimatan	signal barb
Poropuntus normani (Smith,1931)	daun Chantaburi	
Barbonymus schwanenfeldii (Bleeker, 1854)	lampam sungai	tinfoil barb
Rasbora sumatrana (Bleeker,1852)	seluang sumatra	common rasbora
Tor tambra (Valenciennes 1842)	kelah bogor	green mahseer
Puntius brevis (Bleeker, 1849)	tebal sisik Indonesia	swamp barb
Notopteridae		
Notopterus notopterus (Pillas, 1769)	selat	bronze feather
Nandidae		
Pristolepis grootii (Bleeker, 1852)	patung belitung	banded leaf fish
Pristolepis fasciata (Bleeker, 1851)	patung banjarmasin	malayan leafish
Channidae		
Channa striata (Bloch, 1793**)**	haruan	banded snakehead
Cichlidae		
Oreochromis niloticus (Linnaeus, 1758)	tilapia	tilapia
Cichla ocellaris (Bloch and Schneider, 1801)	raja	peacock bass
Bagridae		
Hemibagrus nemurus (Valenciennes 1840**)**	Baung Besar Kalimantan	Java catfish

Table 2.1 continues

Osphronemidae		
Osphronemus goramy (Lacepede, 1801)	kalui Indonesia	giant gourami

2.4 Freshwater fish parasites

According to Goater *et al.* (2014), parasites are living organisms that live on or in another living organism (host) deriving nutrients at the host's expense. Fishes are host to obligate and opportunistic parasites, but obligate parasites are the ones that causes most infections in freshwater fishes (Rajiv & Yahaya, 2015)

Based on the size, parasitic organisms can be classified into macroscopic and microscopic parasites. Helminths and arthropods are macroscopic parasites because they are multi-cellular organisms. In fish parasitic surveys for microscopic parasites, protozoans are only considered (Poulin, 2011). Furthermore, in terms of location, parasitic organisms are also divided into internal parasites and external parasites. Parasites found on the outer surface like the gills, skin, or fins are external parasites while those that lived inside body cavities tissues or organs are internal parasites (Poulin, 2011). Fish parasites can cause severe infection, leading to mortality or exposing the fish to predators (Sures, 2008). Lagrue & Poulin (2015), explained that the effects of fish parasites include castration, muscle degeneration, liver dysfunction, and distortion of the fish body.

One of the most abundant and common freshwater fish parasites is monogenean (Bellay *et al.*, 2015). The parasite species are dominated by parasitic monogeneans that infect cyprinids fish families, in the reservoirs. Other fish parasite groups found in freshwater fish are protozoans, trematodes, cestodes, nematodes, acanthocephalans, and crustaceans (Robert, 2012).

2.4.1 Protozoans

These groups of organisms are microscopic and are all unicellular. Pathogenicity of parasitic protozoans to fish is mostly caused by their capacity to multiply in or on their host (Martins *et al.,* 2015). In the kingdom Protozoa, over 100,000 species of protozoans have been described, with about two-thirds of these being non-parasitic species and the rest symbiotes, commensals and parasitic (Zajac *et al*., 2021).

Several phyla of parasitic protozoans cause infections in freshwater fish and consist of the Sarcomastigophora, Ciliophora, Microspora and Apicomplexa (Zajac *et al*., 2021). White spot disease or ich caused by *Ichthyophthirius multifilis* (Figure 2.2) is one of the most pathogenic ciliate of freshwater fish. Many freshwater fish species are parasitized by *I. multifilis*. The first outbreak of this disease was reported from China (Dickerson, 2012), but it is now a worldwide pathogen. Trophont, tomite and theront are the 3 stages of the life cycle of *I. multifilis* (Dickerson & Findly, 2014). Asexual reproduction takes place in the other stages which are non-parasitic, the theronts infect new fish host (Dickerson, 2012). White spots on the eye, skin, fins, gills, and mouth cavity are the clinical symptoms of *I. multifilis* infection.

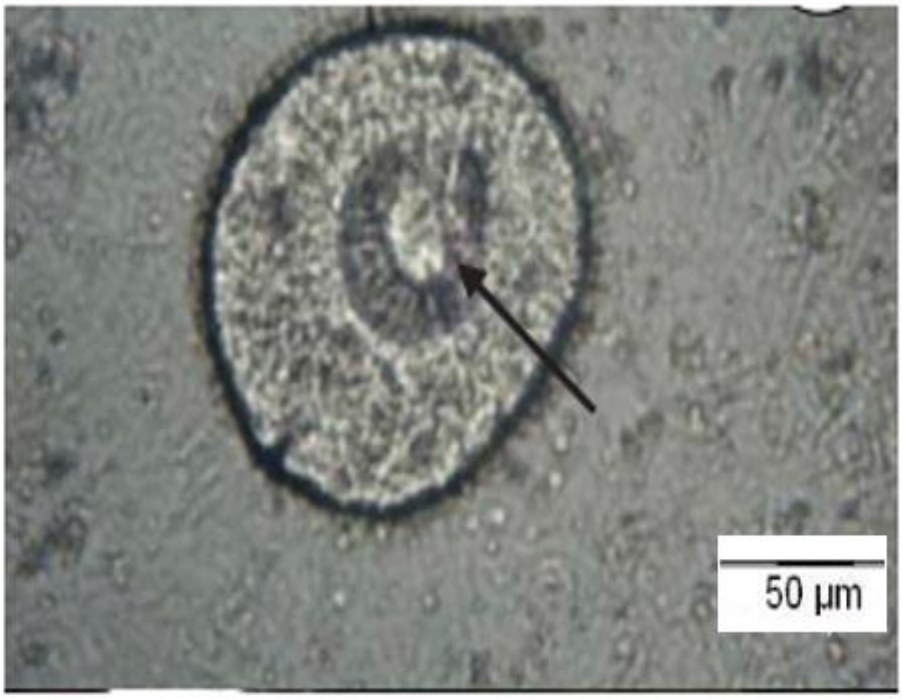

Figure 2.2 *Ichthyophthirius multifilis*, C-shaped macronucleus (arrow) in a mature trophont, scale bar = 50 µm (wet mount), adapted from Mamum *et al*.(2020).

Another common parasitic protozoan of freshwater fish is trichodinid (Tantry *et al*., 2016). This ciliated protozoan is distributed throughout the globe and its host specificity is low (Zajac *et al*., 2021). The main feature of trichodinid (Figure 2.3) is that a slender membrane-enclosed the cup shape body, adoral ciliary spiral surrounds the membrane, possesses a horseshoe-shaped macronucleus (Basson & Van As, 2006). The adhesion, crawling, feeding of trichonids cause destruction of the epithelial tissue of the skin and gill (Tantry *et al*., 2016).

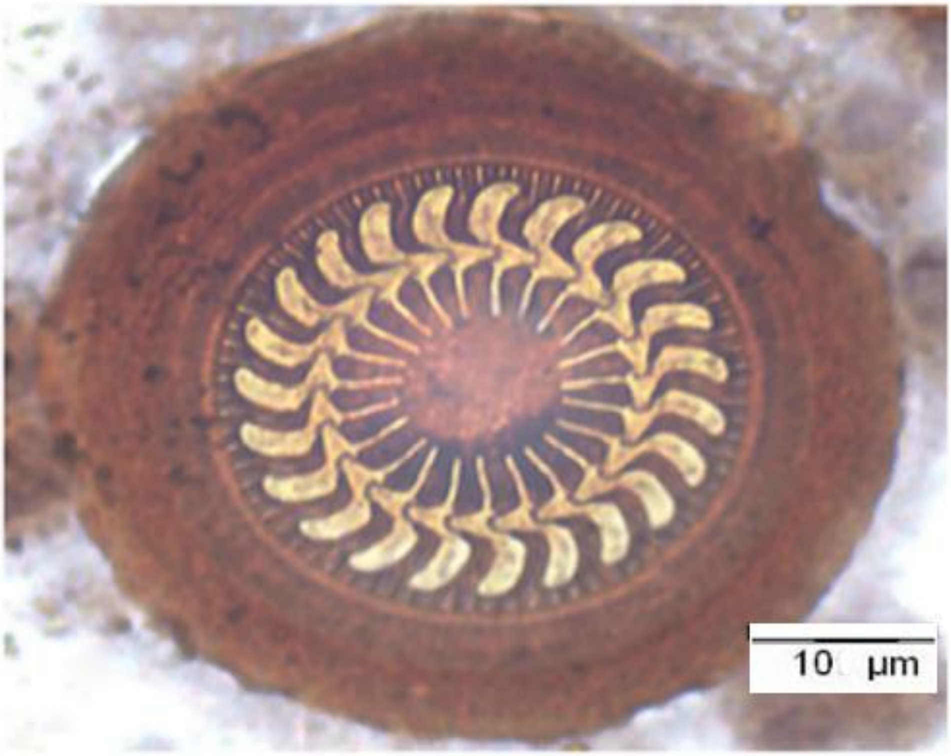

Figure 2.3 Adhesive disc of *Trichodina modesta*, impregnated with silver nitrate, scale bar = 10 µm. Adapted from Basson & Van As, (2006).

Balantidiasis caused by *Balantidium* sp. (Figure 2.4) is a large ciliate protozoan of the intestinal lumen with a large macronucleus (Sun *et al.*, 2017). The body is anteriorly compressed and has an oval shape, the arrangement of cilia is in longitudinal rows, forming a winding spiral at the posterior. At the anterior part of the organism, a slit-like peristome is located. *Balantidium* spp. and *Stentoropsis* spp. are the two members of the family Balantidae that are commonly parasitic in freshwater fish (Sun *et al.*, 2017). These parasites cause enteric diseases, destruction of the epithelium layer of the gut, causing extensive ulcer and granuloma formation when the parasites penetrate deeper into the tissue (Sun *et al.*, 2017).

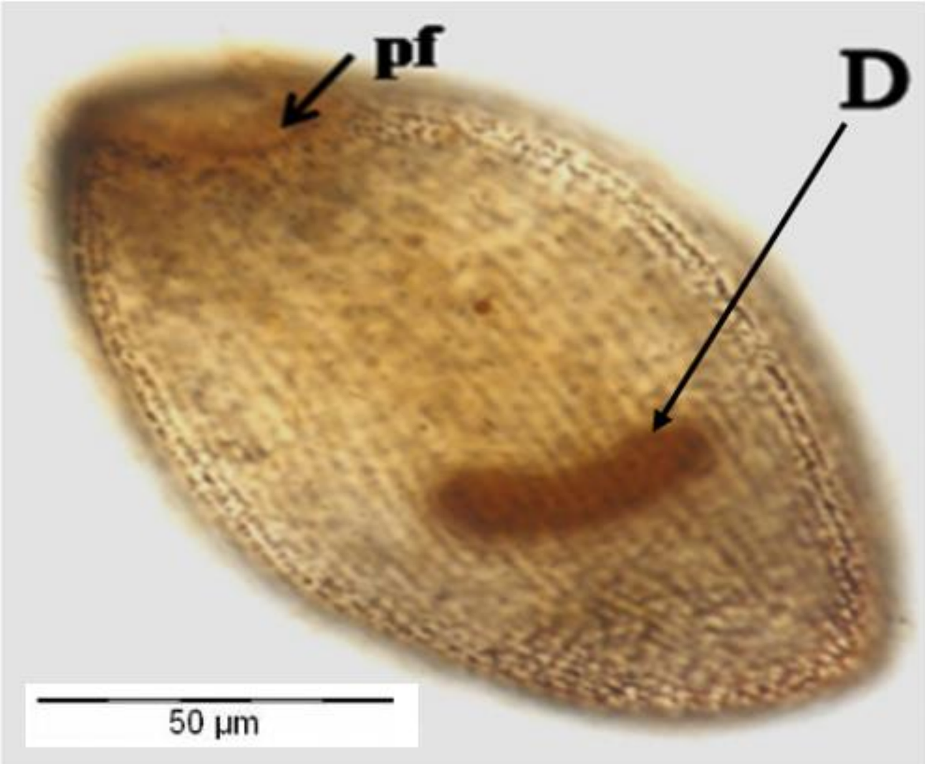

Figure 2.4 Light microscopy images of *Balantidium ctenopharyngodoni*, peripheral fibres (pf), macronucleus (D), Scale bar = 50 µm. Adapted from Sun *et al.*, (2017).

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Despite the presence of *Epistylis* spp. (Figure 2.5), *Zoothamnium* spp., *Chilodonella* spp., *Ichyobodo* spp., and *Piscinoodinium* spp. in fish culture in Malaysia, there is little information on distribution, prevalence rate, or intensities of these parasitic protistans (Lio-po & Lim, 2014).

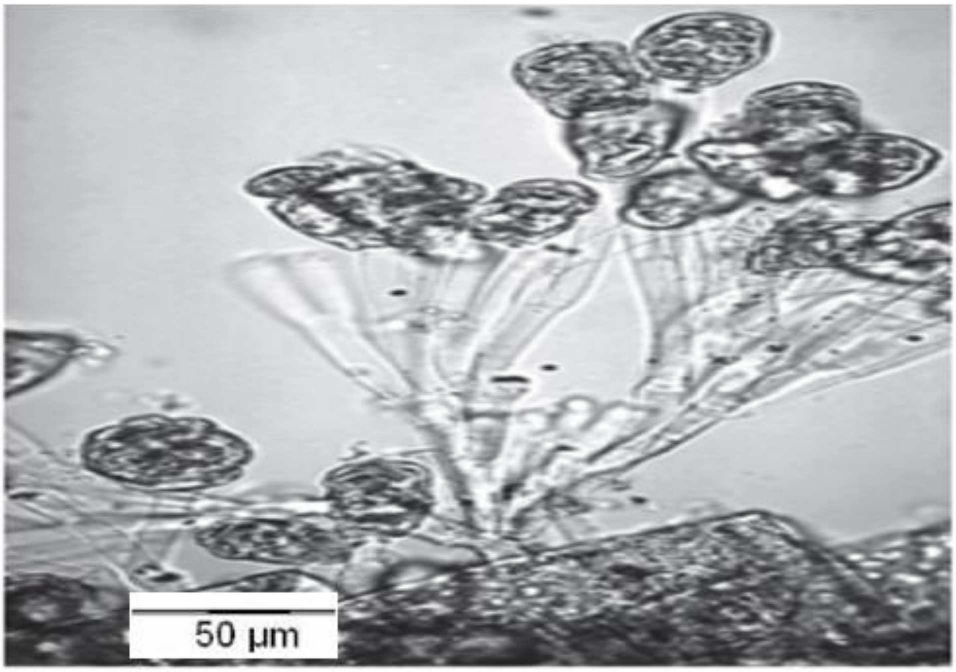

Figure 2.5 Colonies of *Epistylis* spp. Scale bar = 50 µm. Adapted from Lio-po & lim (2014).

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2.4.2 Myxozoa

Phylum Myxozoa belong to the metazoan parasites as the spores comprise many cells that were once classified as parasitic protozoan (Noga, 2010). Myxozoa contains 58 genera and more than 2200 species have been identified, which belong to two classes, the Myxosperea and the Malacosporea (Noga, 2010).

In Malaysia, about 19 species of Myxozoa have been identified which are one *Thelohanellus* spp., nine *Myxobolus* spp. (Figure 2.6), three *Hennegoides* spp., and six *Henneguya* spp., that were isolated from feral and farm fish (Molnár *et al*., 2006; Szekely *et al.*, 2009). The life cycle in some myxozoans is direct while many have an indirect life cycle that involves oligochaete and fish hosts. Myxozoans infect the nervous system, internal organs, gills, skin and muscle forming cysts, lesions when the cysts rupture can lead to the death of the host fish (Noga, 2010).

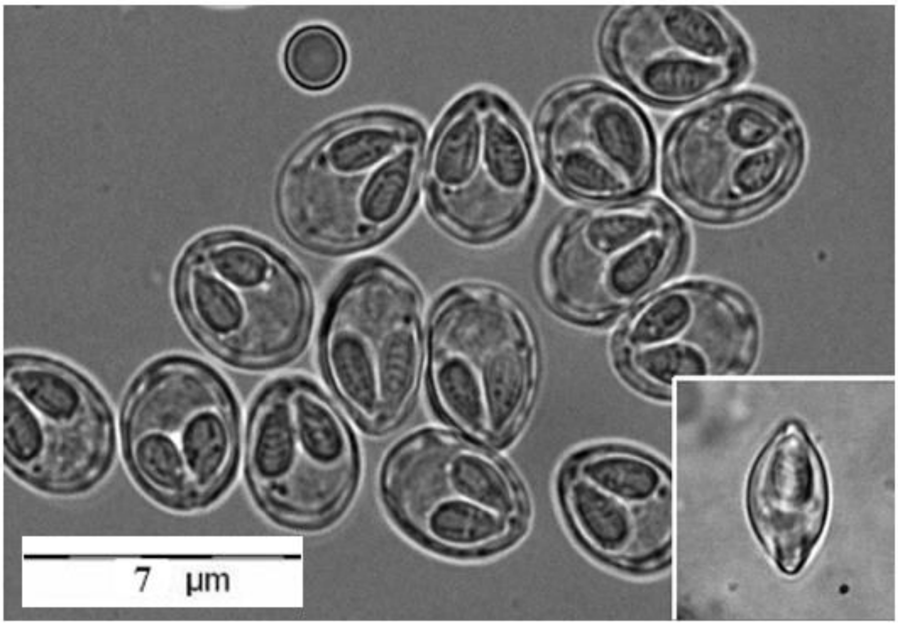

Figure 2.6 *Myxobolus* sp. Spores from cysts on the scales of gray mullet. Scale bar=7 µm. Adapted from Kim *et al*., (2013).

2.4.3 Monogenean

One of the most common parasites of freshwater fish is monogeneans (Bellay 2015). The majority are external parasitic flatworms with direct life cycles. They are mostly host specific and cause infections on the outer body surface of fish, gills and buccal cavity (Zojac *et al.,* 2021). Opishaptor is an adhesive apparatus that is in the posterior part of monogenean which is the most important morphological feature in recognizing monogeneans. Polyopisthocotylea and Monopisthocotylea are the two main classes of monogenean that differ in physiological, reproductive and morphological features (Buchmann & Bresciani, 2006; Reed *et al.,* 2012). Polyopisthocotylean feeds on blood and monopisthocotylean feeds on epithelial of the skin of fish (Perkin *et al.,* 2010).

Dactylogyrus spp. and *Gyrodactylus* spp. are the common genera of parasitic monogeneans on freshwater fishes worldwide. *Dactylogyrus* spp. (Figure 2.7) are mainly parasitic on gill and are oviparous, while *Gyrodactylus* spp. are parasitic on gill, fins, skin and are viviparous (Mayer & Donnelly, 2013). Two hundred species of monogeneans have been identified in Peninsular Malaysia from 25 species of freshwater fishes, which show that only 10-20% of parasitic monogeneans are known (Kritsky, 2007)

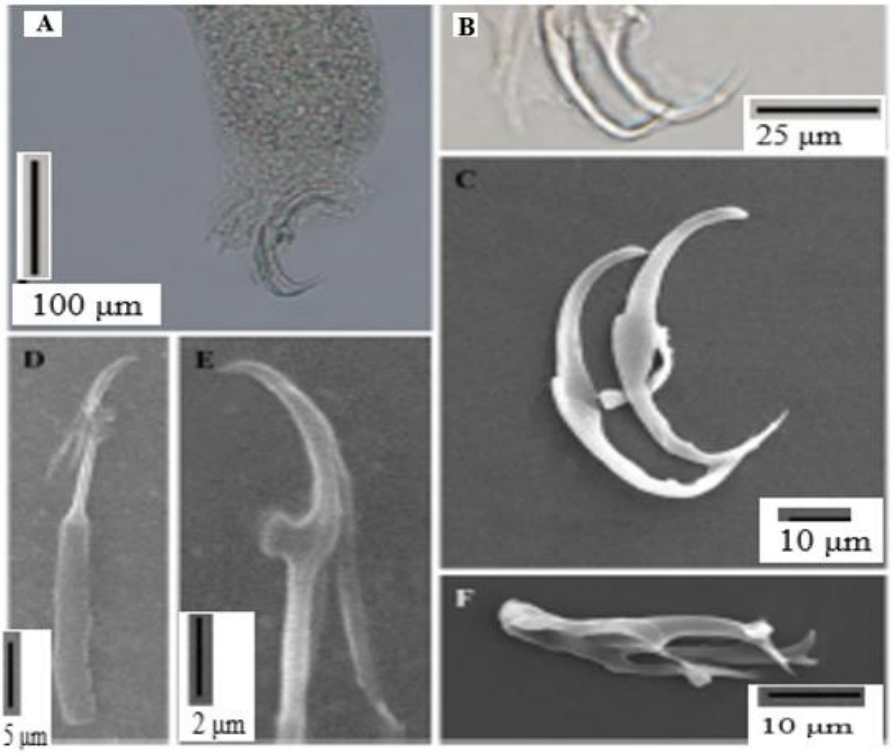

Figure 2.7 *Dactylogyrus formosus* from goldfish collected from Henan province of central China.A: Light micrograph of whole parasite in ventral view; B:light micrograph of the opisthaptoral central hook complex; C: scanning electron micrograph of the opisthaptoral central hook complex; D: scanning electron micrograph of a marginal hook; E:scanning electron micrograph of the marginal hook stickle; F:scanning electron micrograph of male copulatory organ. Scale bars A: 100 µm, B: 25 µm, C: 10 µm, D: 5 µm, E:2 µm, and F:10 µm. Adapted from Tu *et al*., (2015).

2.4.4 Trematoda

Digeneans are flatworms (Figure 2.8) which are internal parasites with many intermediate hosts in their life cycle and mollusk is the first intermediate host, where asexual reproduction of the successive trematodes occurs (Hoffman, 2019). The alimentary canal, body cavities, or blood vessels of vertebrates are the most preferable location of adult trematode for parasitism.

Fish may serve as either paratemic, definitive, or intermediate host. The ileum of fish is the preferred location for adult trematode. They cause less harm to the fish, although with some exceptions, and adult trematodes are host-specific (Parperna & Dzkowski, 2006; Hoffman, 2019). The larval metacercariae of trematodes are pathogenic, not host-specific, cause slow growth as well as a mean of infecting definite host (Parperna & Dzkowski, 2006).

Infection of the fish host by larval trematode is through penetration by the active cercarial (Hoffman, 2019), and successful infestation depends on the cercariae behavior. After cercarial penetration, the metacercaria induces the host to produce an inflammatory reaction that encloses the metacercaria cyst (Parperna & Dzkowski, 2006). The location and number of cysts influence the effect of the severity of the infection. Cysts caused necrotic of the tissue that surrounds them. The trematodes cysts cause damage to the gill of fish which is more severe in juvenile fish (Hoffman, 2019). Adult trematodes have an oval body shape with a spiny or rough surface, anterior and posterior suckers used for movement and attachment (Hoffman, 2019).

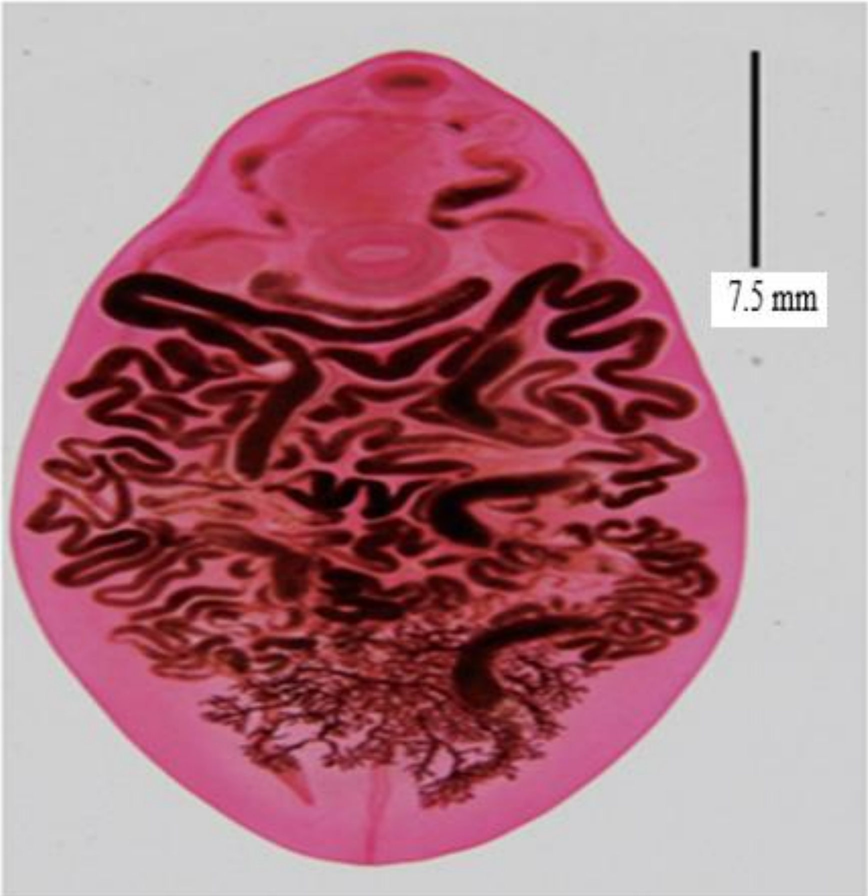

Figure 2.8 *Isoparorchis* sp. (Digenea: Isoparorchiidae) ((Semichon’s acetocarmine stained). Scale bar = 7.5 mm. Adapted from Sohn & Na, (2018).

2.4.5 Cestoda

Tapeworms are flatworms that lack a body cavity, alimentary tract, and elongated tape-like shape. Cestodes' life cycle involves cyclopoid copepods as the intermediate host and they are all endoparasites (Robert, 2012). Adult or larval stages of cestodes infect most fish species. Larva tapeworm stages are found in fish muscle and various internal organs while the adults are in the alimentary tract. Larva cestodes are less host-specific than the adult (Jensen & Bullard, 2010). Adult tapeworms in the alimentary track cause retarded growth, reduced absorption of digested food nutrients, physical damage to the gastrointestinal tract, and migrating larvae cause more pathology in the fish host (Jensen & Bullard, 2010).

Bothriocephalus acheilognathi, Asian tapeworm (Figure 2.9) was described originally from grass carp in China and Japan. The adult parasitizes the alimentary canal of fish (Robert, 2012). The introduction of bighead carp, silver carp and grass carp to aquaculture in Peninsular Malaysia has transmitted Asian tapeworm to the feral fish (Lio-Po & Lim, 2014).

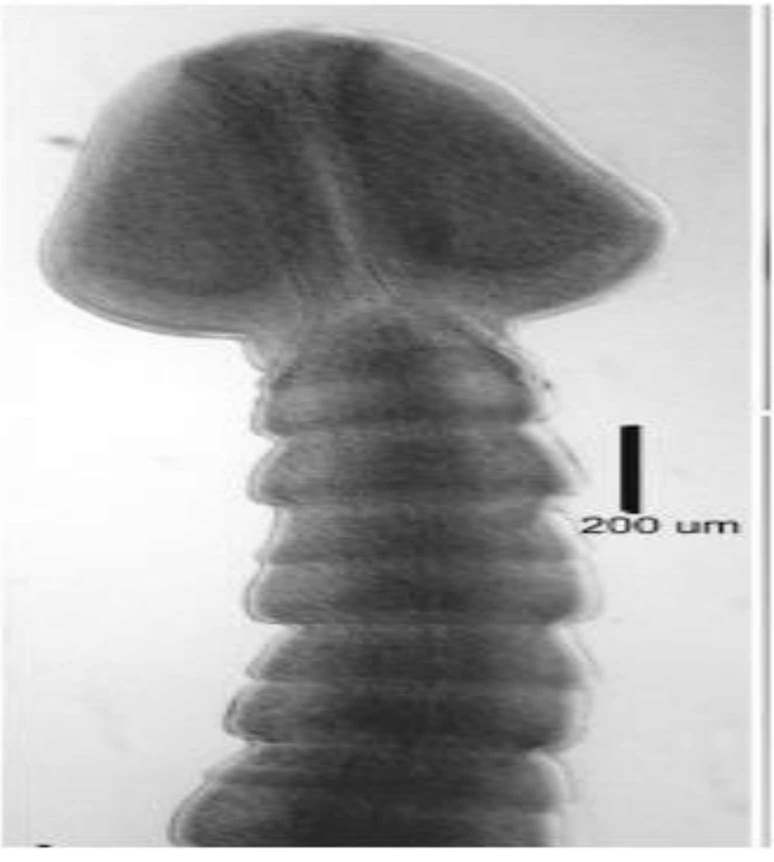

Figure 2.9 *Bothriocephalus acheilognathi*. Anterior portion. Scale bar = 200 μm. Adapted from Choudhury *et al.*, (2013).

2.4.6 Nematoda

The nematodes (roundworms) are cylindrical (Figure 2.10), with elongated bodies made of solid cuticle that tapers at both ends (Hoffman, 2019). They are endoparasitic organism with an indirect life cycle that involves single or many paretenic hosts and one intermediate host; but some species of nematode (*Camallanus coti*) have a direct life cycle (Hadfield, 2021).

The nematode alimentary canal consists of an anterior mouth, esophagus, and intestine (Hoffman, 2019). Adult parasitic nematode usually infects the digestive tract and the larva infect both alimentary canals as well as other organs. Parasitic nematode may use fish as paratenic, intermediate or definitive host (Hadfield, 2021). Nematodes are dioecious organisms and adult males, and females have unique morphological features that are used in identification (Hoffman, 2019).

In Asia and South America, eating of undercooked or raw fish by a human can lead to parasitic infection by the larval stage of *Gnathostoma* spp. which causes larva migrans syndromes (Diaz, 2015) or capillariasis cause by *Capillaria philipinesis* that is widespread in South-East Asia countries (Hoffman, 2019), which may lead to severe diarrhea and abdominal pain. In fish, the nematodes harm the fish host by fixing to the wall of the alimentary canal, migrating, or developing in them, feeding on the host blood, tissues, and digested foods (Hoffman, 2019).

Figure 2.10 Juvenile *Camallanus* nematode from a fire mouth cichlid. Adapted from Yanong, (2003). Scale bar= 750 µm.

2.4.7 Acanthocephalans

One of the main features of acanthocephala or “spiny-headed’ worm (Figure 2.11) is the possession of many rows of recurved hooks that crown the evaginable proboscis. They lack gastrointestinal track and both male and female sexes are separates. The shape of the worms resembles a sack-like structure, proboscis connected to the lemnisci and sex organs located at the posterior part of the organism (Robert, 2012).

The development of all acanthocephalan requires one or many intermediate hosts. The adult is in the alimentary canal where they lay eggs. Amphipods, copepods or isopods are the first intermediate host of fish acanthocephala, where the acanthela (first larva) hatch and when consumed by the definitive host, it grows to the adult stage (Robert, 2012). When fish serve as a definitive host for adult acanthocephala, the parasite is harbored in the digestive tract, or as an intermediate host, the cystacanth is found in tissues. Predatory fish are the definitive host of acanthocephalans (Hoffman, 2019). The distribution of acanthocephalan is global in both fresh and marine water (Nickol, 2006).

Diseases cause by acanthocephalan are due to the attachment of the adult parasite in the ileum and the encystment of the larva in the tissue of the host. The infection depends on the penetration of the proboscis into the host tissue when the attachment is on the epithelial tissue of the mucosa, the pathological effect is minimal (*Acanthogyrus* spp. and *Acanthocephalus* spp.), but where attachment of the proboscis is in the muscular tissue of the intestine, or pierce the small intestine (*Pomphorhynchus* spp.), it causes fibrosis and granuloma (Robert, 2012).